INFLUENCE OF HYPOPHYSECTOMY ON THE PANCREATIC DIABETES OF DOGFISH

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Using toads and dogs, Houssay and Biasotti (1930*a*, 1930*b*) have shown the marked influence of hypophysectomy on the course of the diabetes produced by pancreatectomy. In their pancreatectomized animals the diabetes was definitely milder when the hypophysis had been removed. It was the purpose of this investigation to determine whether or not the general conclusions reached by these investigators were applicable to a lower form. The smooth dogfish (*Mustelus canis*) was chosen on account of its availability and its position in the vertebrate scale. Furthermore, its cartilaginous skull renders hypophysectomy a comparatively simple procedure.

Pancreas, Hypophysis, and Glycemia in Elasmobranch Fishes

Elasmobranchs have a large pancreas, its tissue being made up of glandular acini and of insular cells functionally equivalent to the islets of Langerhans in the pancreas of mammals (Jackson, 1922). The isolation and removal of the entire pancreas are procedures easily performed without any serious bleeding. Herring (1911) has shown that the hypophysis of elasmobranchs is developed almost entirely from Rathke's pouch. This same investigator (Herring, 1913) states that "the elasmobranch pituitary differs from all other pituitaries in not possessing a posterior lobe. The brain wall of the embryo merely evaginates to form a paired saccus vasculosus, but no pars nervosa is formed." And although de Beer (1926) showed that there is an extension of neuroglia fibers from the border of the infundibular cavity which penetrates the posterior lobe (pars intermedia), it is certainly true that the selachian lacks a true pars nervosa. This means that one has to deal in this form with a hypophysis composed of (*a*) an anterior lobe of eosinophile and basophile cells, (*b*) a posterior lobe (pars intermedia) of basophile cells, and (*c*) a ventral lobe composed also of basophile cells, but containing, in addition, certain curious large cells staining with eosin and of undetermined significance (de Beer, 1926). In the hypophysectomies of the present investigation

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no attempt was made to discriminate between these various parts. All removals of the gland have been total, and were performed according to the technique described by Lundstrom and Bard (1932).

The normal glycemia in fish has not yet been satisfactorily worked out. All the recent studies dealing with the blood sugar of fishes have shown great discrepancies between the values found not only among specimens of different species, but also among different individuals belonging to the same species. Although no one has been able to determine precisely all the factors responsible for these discrepancies, undoubtedly the variable degrees of asphyxiation involved in the process of obtaining the blood samples for analysis stand as a cause of paramount importance in explaining the widespread variability of results. There is no doubt that asphyxia induces a condition of hyperglycemia which may last during a period of several days even when the fish is replaced in sufficiently oxygenated water. This has been shown by McCormick and Macleod (1925), Simpson (1926), and Kisch (1929). The asphyxial blood-sugar rise is due to a mobilization of glycogen from the liver (Simpson, 1928; Kisch, 1929).

Apart from the influence of asphyxia, the blood-sugar level seems to vary with the different species of fish because of the differences in their habits of life. According to the investigations of Gray and Hall (1930), who studied fifteen species of teleosts, fast-swimming fishes depending on the speed of their movements to catch their prey have a higher blood-sugar level than those less active bottom-feeders that live on crustaceans and other slow-moving creatures.

Before proceeding to the main problem, an attempt was made to determine the normal blood-sugar level of *Mustelus canis*. Blood samples were directly withdrawn from the heart by means of a syringe with the needle inserted through the ventral median line at the anterior edge of the pectoral girdle. As a rule 0.5 cc. of blood was used for each determination. The reducing substances of blood after precipitation of proteins were determined by the Shaffer and Hartmann method (1921) and computed as glucose.

Blood-sugar determinations were performed on each fish used (total of 39) in this work immediately after its arrival in the laboratory from its place of capture in traps situated out in the ocean. The values obtained ranged between 72 and 250 milligrams of glucose per 100 cc. of blood. Sometimes very low values were recorded (from 0 to 50 milligrams per cent), but animals with such low blood-sugar figures were either moribund, or, if apparently normal, they invariably died within a few hours. In this respect our observations entirely agree with those of Scott (1921). After this initial blood-sugar determination, in order to let the fish recover from the effects of asphyxia and rough handling incident to its capture, it was placed in an immersed floating cage, several meters off shore, exposed to tides and marine currents. After 48 hours, a new sample of blood was withdrawn as quickly as possible and the amount of sugar determined. The figures thus obtained in 10 specimens of *Mustelus canis* ranged between 65 and 137 milligrams of glucose per 100 cc. of blood, the average being 105 ± 5 milligrams, and the standard deviation ± 22 milligrams. No food was given to the animals.

These results, as well as the data found in the literature regarding the blood-sugar level of elasmobranchs, seem to show a clear difference between the amount of glucose in the blood of the species of the genus *Mustelus* (smooth dogfish) as compared with that in the blood of species of the genus *Squalus* (spiny or horned dogfish). In effect, the "weighed average" of all the analyses on fish of the genus *Mustelus* reported by Fandard and Ranc (1914), Scott (1921), Menten (1927) and Fremont-Smith and Dailey (1932), turns out to be 99 milligrams of glucose per 100 cc. of blood, for a total of 34 animals examined. Denis (1922), omitting to say how many animals she investigated, reports for the blood of *Mustelus canis* amounts of glucose ranging from 80 to 181 milligrams per 100 cc., the majority of her results falling between 90 and 110 milligrams.

In so far as the genus *Squalus* is concerned, the "weighed average" of the analyses performed by Claude Bernard (1877), Lang and Macleod (1920), and White (1928), is of the order of 36 milligrams of glucose per 100 cc. of blood for a total of 15 animals examined. It is impossible to account for this difference on the basis of the view that the difference in the blood-sugar levels of these two genera is related to their different habits of life in the way pointed out by Gray and Hall for teleosts. While according to Bigelow and Welsh (1924), the spiny dogfish (*Squalus*) is a strong, fast-swimming animal, the smooth dogfish (*Mustelus*) is a bottom fish, feeding principally on crustaceans. Elasmobranchs of the genera *Torpedo* and *Scyllium*, which according to the analyses of Diamare (1905 and 1906), Diamare and Montuori (1907), and Kisch (1929) have also lower amounts of blood sugar than *Mustelus*, are slow-moving animals of the sea bottom (Couch, 1868).

Pancreatic Diabetes in Fish

Capparelli (1894) was apparently the first to study the effect of pancreatectomy on fish. He removed the pancreas from eels, and was able to find marked glycosuria as a consequence. Diamare (1905, 1906, and 1911), working on elasmobranchs, found considerable amounts of sugar in the blood of *Scyllium* and *Torpedo* after removal of the pancreas, whereas he was unable to detect any sugar in the blood of these animals before the operation. Probably Diamare and subsequently Diamare and Montuori (1907) failed to find sugar in the normal blood of *Scyllium* and *Torpedo* because of the inadequate methods available at that time, as they themselves suggested. More recently McCormick and Macleod (1925) found in *Myoxocephalus* (sculpin) marked hyperglycemia as a consequence of the ablation of the principal islets, easily removed in this animal, leaving intact the remaining pancreatic tissue. Simpson (1926) working on *Myoxocephalus* and *Ameiurus* confirmed the observations of McCormick and Macleod.

Experiments and Results

Limiting the present investigation to animals of the same species and following as uniform a procedure as possible in handling the animals before, during, and after the operations, it is possible to a certain extent to make "constants" out of the several factors, known and unknown, which modify the blood-sugar level, aside from the experimental conditions created for purpose of the study (pancreatectomy, hypophysectomy, etc.). Finally, a statistical treatment of the data obtained will enable us to get a more complete idea of the significance and validity of the differences between the average values obtained under the different experimental conditions (Dunn, 1929).

Medium-sized animals were chosen (from 70 to 90 centimeters long), regardless of sex, but pregnant females were rejected. Blood-sugar determinations were always performed by Shaffer and Hartmann's method (1921).

The first step of our procedure was always to withdraw a sample of blood from the heart as previously described, for a blood-sugar determination. Then the necessary operations were performed, with care to avoid asphyxia as much as possible. A constant flow of sea water was maintained through the mouth and gills and under these circumstances the respiratory movements proceeded in normal fashion.

The pancreas was removed through an abdominal incision about three centimeters long and the abdominal wall was subsequently closed in layers by silk sutures. The hypophysis was extirpated through a buccal approach. No anesthetic was used. The longest operation lasted about twenty minutes. After they had been operated the animals were treated as previously described.

Forty-eight hours after operation a new sample of blood was secured and another blood-sugar determination performed. The forty-eighth hour after operating proved to be a critical juncture.

TABLE I

Glycemia of Dogfish under Different Experimental Conditions
(Glucose in milligrams per 100 cc. of blood)

OPERATION	Laparotomy		Hypophysectomy		Pancreatectomy		Hypophysectomy and Pancreatectomy		Pancreatectomy and Injury of the Hypothalamus	
	Before operation	After 48 hours	Before operation	After 48 hours	Before operation	After 48 hours	Before operation	After 48 hours	Before operation	After 48 hours
Individual Results	83	150	144	100	185	431	165	155	115	431
	247	125	185	65	144	350	175	328	183	431
	220	115	170	370	144	428	115	267	203	430
	185	185	100	185	205	370	175	370	162	392
	160	105	160	95	124	431	225	205	144	423
	72	105	144	115	195	368	267	395		
	120	185	142	117	155	380	124	349		
	122	226	155	137	135	430	115	215		
					150	430	137	307		
Averages	151±13	149±11	151±6	148±23	159±6	402±7	166±11	288±18	161±10	421±5
Stand. dev.	±52	±42	±23	±90	±26	±32	±49	±77	±30	±15
Prob. error	±35	±28	±15	±60	±17	±21	±33	±52	±20	±10
Differences	2±16		3±23		243±9		122±21		260±11	

Before that time the effects of asphyxia and rough handling inherent in the operations were still too marked, and beyond the 48 hours the mortality began to be rather high.

The animals fall into five groups. The results show respectively the separate effects on blood sugar of (*a*) simple laparotomy, (*b*) hypophysectomy, (*c*) pancreatectomy, (*d*) pancreatectomy and hypophysectomy, and (*e*) pancreatectomy and injury of the hypothalamic region of the brain. The operations were performed in such a way as to permit having animals of different groups simultaneously exposed to the same environmental conditions.

As Table I shows, neither the laparotomy alone, nor the simple hypophysectomy, exerted any significant influence on the glycemia. The values, of course, were above those considered normal, but of the same order of magnitude as the values found before the operation. The removal of the pancreas, as was to be expected, caused a marked increase of blood sugar values: from 159 ± 6 milligrams of glucose per 100 cc. of blood as the average for 9 unoperated animals, to 402 ± 7 milligrams per 100 cc. of blood as an average for the same animals 48 hours after the operation.

When both pancreas and hypophysis had been removed in the course of the same operation, a condition of hyperglycemia also ensued, but the average for this whole group as well as the individual figures were lower than those encountered when the pancreas alone was taken out. That this difference ($- 114 \pm 19$ milligrams) which is certainly significant, is due to the absence of the pituitary body and not to the influence of some direct nervous factor brought into play by the operative traumatism, is demonstrated by the fact that in animals of the fifth group in which the pancreas was removed, leaving intact the hypophysis but injuring the adjacent nervous tissue (hypothalamus), the blood-sugar values were even higher than those found when the pancreas alone was extirpated.

Shortage of time and animals prevented the study of the action of pituitary grafts and the action of pituitary extracts, but the data here reported support the conclusion that in the dogfish (elasmobranch fish) just as in the toad (batracian) or in the dog (mammal) the hypophysis exerts an aggravating influence on pancreatic diabetes, the mechanism of which is still obscure.

Summary

Laparotomy or hypophysectomy does not change the blood-sugar level in the dogfish. Pancreatectomy produces a marked hyperglycemia. The hyperglycemia is, however, less marked if pancrea-

tectomy is accompanied by hypophysectomy, but it is slightly more marked if in addition to pancreatectomy the hypothalamus is injured.

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